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Original Article

Article title: Increasing stomatal conductance in response to rising atmospheric CO₂

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Running title: Stomatal conductance and elevated CO₂
ABSTRACT

Background and Aims: Studies have indicated that plant stomatal conductance ($g_s$) decreases in response to elevated atmospheric CO$_2$, a phenomenon of significance for the global hydrological cycle. However, $g_s$ increases across certain CO$_2$ ranges have been predicted by optimisation models. The aim of this work was to demonstrate that under certain environmental condition, $g_s$ can increase in response to elevated CO$_2$.

Methods: When using (i) an extensive, up-to-date, synthesis of $g_s$ responses in FACE experiments, (ii) in situ measurements across four biomes showing dynamic $g_s$ responses to a CO$_2$ rise of ~50ppm (characterising the change in this greenhouse gas over the past three decades) and (iii) a photosynthesis-stomatal conductance model, it is demonstrated that $g_s$ can in some cases increase in response to increasing atmospheric CO$_2$.

Key Results: Field observations are corroborated by an extensive synthesis of $g_s$ responses in FACE experiments showing that 11.8% of $g_s$ responses under experimentally elevated CO$_2$ are positive. They are further supported by a strong data-model fit ($r^2=0.607$) using a stomatal optimization model applied to the field $g_s$ dataset. A parameter space identified in the Farquhar-Ball-Berry photosynthesis-stomatal conductance model confirms field observations of increasing $g_s$ under elevated CO$_2$ in hot dry conditions. It was shown that contrary to the general assumption, positive $g_s$ responses to elevated CO$_2$, although relatively rare, are a feature of woody taxa adapted to warm, low-humidity conditions, and that this response is also demonstrated in global simulations using the Community Land Model (CLM4).

Conclusions: The results contradict the over-simplistic notion that global vegetation always responds with decreasing $g_s$ to elevated CO$_2$, a finding that has important implications for predicting future vegetation feedbacks on the hydrological cycle at the regional level.

Key words: Stomata, stomatal conductance, climate change, CO$_2$, hydrology, CLM,
vegetation, run-off, drought, photosynthesis, temperature, VPD

INTRODUCTION

Water loss through plant stomata- small pores on the surface of leaves through which gas exchange between plants and the atmosphere takes place - is an unavoidable trade-off in the exchange for CO₂, the substrate for photosynthesis. Decreased stomatal conductance (gₛ), via physiological (stomata responding dynamically to environmental stimuli) and/or morphological changes (via alteration in stomatal density and size) has been observed in elevated carbon dioxide (CO₂) environments in both laboratory and Free Air CO₂ Enrichment (FACE) studies (Ainsworth and Rogers 2007; Drake et al. 1997; Farquhar and Sharkey 1982; Leuzinger and Körner 2007; Woodward 1987). However, recent studies suggest that rising atmospheric CO₂-induced decreases in gₛ may be offset by contemporaneous increases of leaf area index (LAI) during the course of a growing season (Frank et al. 2015; Niu et al. 2013; Piao et al. 2007; Schymanski et al. 2015; Wu et al. 2012). Thus, despite significant improvements in our understanding of plant-atmosphere interactions in recent years, the net stomatal conductance response of the entire global vegetation system to rising anthropogenic CO₂ remains unclear.

In addition, little is known regarding the physiological response of plants to increasing CO₂ across multiple biomes, and in varying temperature and humidity regimes. For example, FACE studies are predominantly limited to the mid-latitudes of the northern hemisphere (Fig. 1), biasing our understanding of plant responses to these regions. Moreover, disparate vegetation responses in dry and drought prone environments have been reported (Choat et al. 2012; De Kauwe et al. 2015; Limousin et al. 2013; Mencuccini et al. 2015; Zhou et al. 2013). It is therefore critical to improve our understanding of these responses in order to better
predict future freshwater cycling, especially in regions vulnerable to drought and
desertification in the 21st century (Lawrence et al. 2011).

Here we demonstrate that $g_s$ can in some cases increase in response to increasing atmospheric
CO$_2$. This is shown using (i) in situ measurements of 51 woody plant taxa across four biomes
showing dynamic $g_s$ responses to a CO$_2$ rise of \sim{}50ppm which represents the change in this
greenhouse gas over the past three decades, (ii) an extensive, up-to-date, synthesis of $g_s$
responses in FACE experiments, (iii) both the stand-alone and Community Land Model
version 4 (CLM4)-integrated application of the Farquhar-Ball-Berry photosynthesis-stomatal
conductance model and (iv) the Medlyn et al. (2011) optimal stomatal model.

MATERIALS AND METHODS

Synthesis of Free Air CO$_2$ Enrichment (FACE) studies

A literature review was undertaken of studies that specifically focused on the effect of
elevated CO$_2$ on plant stomatal conductance ($g_s$) in FACE experiments. A total of 51 studies
were included in the database (Adachi et al. 2014; Ainsworth and Rogers 2007; Ainsworth et
al. 2003; Bader et al. 2010; Bhattacharya et al. 1994; Borjigidai et al. 2006; Bryant et al.
1998; Calfapietra et al. 2005; Chen et al. 2014; Ellsworth 1999; Ellsworth et al. 1995;
Ellsworth et al. 2012; Garcia et al. 1998; Ghini et al. 2015; Grant et al. 1999; Gunderson et
et al. 1994; Hileman et al. 1992; Huxman and Smith 2001; Ji et al. 2015; Keel et al. 2006;
Leakey et al. 2006; Lee et al. 2001; Marchi et al. 2004; McElrone et al. 2005; Naumburg and
1997; Noormets et al. 2001; Nowak et al. 2001; Pataki et al. 2000; Pearson et al. 1995;
Rogers et al. 2004; Ruhil et al. 2015; Shimono et al. 2010; Singsaas et al. 2000; Tricker et al. 2005; Wall et al. 2000; Wall et al. 2001; Wechsung et al. 2000; Wullschleger et al. 2002; Yoshimoto et al. 2005). The FACE synthesis was built on the original data set by Ainsworth and Rogers (2007). Values reported in tables and in the text were taken directly from publications, whereas results in graphs were digitized. Individual independent observations were obtained following the longest period of CO₂ exposure reported in each study (independent = plant; repeated = species). Studies that examined multi-factorial designs could have contributed several observations for each response variable (e.g. drought, nitrogen enrichment etc.). The mean, standard deviation (SD) and the effect size of the treatment (Ne) and relative control treatment (Na) were recorded. If standard error (SE) was reported we transformed these according to SE=SD*[(n-1)/2]. Database records typically included the year and month the data were collected, GPS site locations, ambient CO₂, elevated CO₂, study organism (incl. varieties), plant functional type (PFT), photosynthetic pathway and other experimental treatments (e.g. nitrogen fertilization). Stomatal conductance measurements from 52 different species, within seven PFTs (C3 crops, C3 forbs, C3 grasses, C3 herbs, C3 shrubs and C3 conifer and C3 broadleaved trees) were included in the analysis. The ranges of ambient and elevated CO₂ between studies were 350-411ppm and 538-680ppm respectively. A kernel density estimation was used to visualise the stomatal conductance data by estimating the unknown probability of the data, based on a sample of points taken from that distribution.

Dynamic gₜ responses to CO₂ change (across four biomes)
Assessment of the dynamic stomatal responses to increasing CO₂ across four different biomes (including a tropical seasonal biome which had been subjected to drought) was achieved during a 10-week scientific expedition to North and Central America in the summer of 2014.
A total of 51 woody tree and shrub species were measured with a CIRAS-2 gas analyser (PP-Systems, Amesbury, MA, USA) attached to a PLC6 (U) cuvette fitted with a 1.7 cm² measurement window and a red/white light LED unit.

Measurements were carried out (Fig. 3) at two boreal forest sites (16 species, Bird Creek [60°58’N, 149°28’W] and Kenai [60°33.3’N, 151°12.8’W], Alaska, USA), one temperate deciduous forest site (11 species, Smithsonian Environmental Research Centre [38°53’N, 76°32’W], Maryland, USA), two tropical seasonal forest (wet) sites (15 species, Cambalache [18°27’N, 66°35’W] and Guajataca [18°24’N, 66°58’W], Puerto Rico) one of which had undergone a long drought period (Cambalache), and one tropical seasonal forest (dry) site (9 species, Guanica [17°93’N, 66°92’W], Puerto Rico). See Table S1 for a complete species list.

Stomatal responses were assessed on an average of four individuals per species between 9:00 am and 13:00 pm. A sun exposed branch was sampled following standard protocol (Berveiller et al. 2007; Dang et al. 1997; Domingues et al. 2010; Koch et al. 2004; Rowland et al. 2015) from each individual using either a pruner (shrubs) or a pole with a scythe fitted on its top (trees) and was immediately recut under water. Following this, a fully expanded leaf from each branch was enclosed in the cuvette of the gas analyser, which was running at a sub-ambient ~year 1990 reference CO₂ concentration of 354ppm (Betts et al. 2016). Stomatal conductance at sub-ambient CO₂ concentration was recorded upon stabilisation of its value, which typically took less than 15 minutes. Subsequently, reference CO₂ was established at 400ppm (year 2016 values) (Betts et al. 2016) and the leaf was left to equilibrate for at least 15 minutes before gs at modern ambient CO₂ was recorded. Randomization of the sequence of the two treatments was ensured; overall about 65% of the measurements started at 400ppm (386.6±0.5) and were reduced to 354ppm (342.4±0.5), while the rest of measurements (35%)
started at 354 ppm and were increased to 400ppm. On several occasions the reversibility of
the CO$_2$ effects on $g_s$ was tested. This was done by measuring $g_s$ at a starting CO$_2$
concentration of 400ppm, after which CO$_2$ was reduced to 354ppm for several minutes,
before it was returned to the initial concentration of 400ppm. The final $g_s$ values at 400ppm
were the same as those initially recorded (data not shown).

Stomatal responses to a subtle increase in CO$_2$ were estimated as the percentage change in the
$g_s$ values between sub-ambient CO$_2$ and modern ambient CO$_2$. Air flow, light intensity and
incoming mole fraction of water during the measurements were maintained at 200 cm$^3$ min$^{-1}$,
1000 $\mu$molm$^{-2}$s$^{-1}$ and 80-90 % of ambient respectively. Since ambient and leaf temperatures
varied significantly between the beginning and the end of the daily measurement time
window in all biomes, the measurements were taken at the calculated mean and biome-
specific leaf temperature at 9:00 am. Calculation was performed early on the first
measurement day at each site by running the gas analyser at the set points mentioned above
(i.e. 1000 $\mu$molm$^{-2}$s$^{-1}$ of light, 80-90 % of ambient water vapour, 400 $\mu$molmol$^{-1}$ CO$_2$, no
temperature control) and by recording the leaf temperatures of at least 10 leaves belonging to
10 different species growing at the site. Differences in $g_s$ responses between biomes were
tested on the normal data using ANOVA analysis. Moreover, a linear model was used to test
for the correlation of $g_s$ to VPD and leaf temperature and the modelled and observed $g_s$ data.
Mixed effects models were used to test which variables best explain the observed changes in
$g_s$ and the best model was selected following Akaike Information Criterion (AIC).

Farquhar-Ball-Berry model (combined photosynthesis and $g_s$)
The model relates $g_s$ to net leaf photosynthesis, scaled by the relative humidity at the leaf
surface and the CO$_2$ concentration at the leaf surface (Collatz et al. 1991; Sellers et al. 1996).
It solves the following three equations:
where \( g_s \) is the stomatal conductance to water vapour, \( A \) is the photosynthetic uptake flux of CO\(_2\), \( c_a \) and \( c_i \) are partial pressures of CO\(_2\) just outside and inside the stomata respectively, \( p_a = 10^5 \) Pa is atmospheric pressure, \( e_a \) and \( e_i \) the water vapour pressures just outside and inside the stomata respectively (the latter computed as the saturation vapour pressure at the leaf temperature \( T_v \)), and \( m \) and \( b \) are empirical constants taken as \( m = 6 \) and \( b = 3 \times 10^4 \) μmol m\(^{-2}\) s\(^{-1}\). The uptake flux is taken to be the minimum of three rate-limiting processes for C\(_3\) plants: Rubisco limitation, \( w_c = \frac{V_{\text{cmax}} \left( c_i - \Gamma^* \right)}{(c_i + K_c + 0.1 K_c / K_o)} \); light limitation, \( w_l = \alpha \frac{\text{PAR} \left( c_i - \Gamma^* \right)}{(c_i + 2 \Gamma^*)} \), and export limitation \( w_e = 0.5 V_{\text{cmax}} \). In these expressions \( K_c \) and \( K_o \) are Michaelis-Menten constants for CO\(_2\) and O\(_2\) respectively which vary with leaf temperature \( T_v \) (expressed in °C) as \( K_c = K_{c25} e^{0.88(T_v - 25)} \) and \( K_o = K_{o25} e^{0.29(T_v - 41)} \) where \( K_{c25} = 30 \) and \( K_{o25} = 30000 \) are reference values while \( a_{kc} = 2.1 \) and \( a_{ko} = 1.2 \). The CO\(_2\) compensation point is taken as \( \Gamma^* = 0.105 \) mPa of oxygen. PAR = 1000 μmol m\(^{-2}\) s\(^{-1}\) is the photosynthetically active radiation flux falling on the leaf, and \( \alpha = 0.06 \) is the quantum efficiency of photosynthesis. Finally, \( V_{\text{cmax}} \) is the temperature-dependent maximum carboxylation rate modelled following Katul et al. (2009) as \( V_{\text{cmax}} = V_{\text{cmax}25} e^{0.88(T_v - 25)} / (1 + e^{0.29(T_v - 41)}) \) where \( V_{\text{cmax}25} = 60 \) μmol m\(^{-2}\) s\(^{-1}\) is the maximum carboxylation rate at 25°C. Given values of \( c_a, e_a, T_v, \) PAR and \( V_{\text{cmax}25} \), the equations are solved numerically using an iterative
method to yield \( c_i, A \) and \( g_s \).

**Optimisation model**

For the comparison of our field data with the optimum \( g_s \) model of Medlyn *et al.* (2011) we used measured values of \( A, c_a \) and VPD and PFT specific \( g_i \) values for evergreen and deciduous species from Lin *et al.* (2015). We assumed that \( g_0 \) was 20 mmol m\(^{-2}\) s\(^{-1}\). The optimal model was as follows:

\[
\frac{g_s}{g_0} = \left(1 + \frac{g_s}{g_0}\right) = \frac{A}{\sqrt{D}} c_a
\]

(4)

where \( D \) is VPD (kPa), \( g_i \) is the model coefficient and \( g_0 \) the minimum \( g_s \) (mol m\(^{-2}\) s\(^{-1}\)). The reader should be aware that this instance of the Ball-Berry model is stand-alone, and not linked to soil moisture through a land model.

**The Community Land Model version 4 (CLM4)**

The Community Land Model version 4 (CLM4), released in 2010 (Lawrence *et al.* 2011; Oleson *et al.* 2010) was used in this study. Land cover and atmospheric weather conditions serve as boundary conditions for CLM4. Grid cells in CLM4 may include vegetation, wetlands, lakes, glacier, and urban regions. CLM4 can be used in conjunction with the other models in the Community Earth System Model (CESM), or independently (stand-alone), as is the case here. This is referred to as an I-compset. Specifically we have used the I-compset with an f19g16 resolution and CLM4 satellite phenology. This simulation has the carbon and nitrogen cycling (biogeophysics “CN”) turned off. CLM4 parameterizes stomatal responses via a Farquhar-Ball-Berry scheme as described above.
CLM4 uses atmospheric boundary conditions for integration. We use the QIAN atmospheric input data set, for 1972–2004 (Qian et al. 2006). This is a global forcing dataset for the period 1948–2004 with 3-hourly temporal and T62 spatial resolution (1.875°). The dataset was developed by combining analyses of monthly precipitation and surface air temperature with intra-monthly variations from the National Centers for Environmental Prediction – National Center for Atmospheric Research (NCEP–NCAR) reanalysis (Qian et al. 2006). Using the Icompset we performed experiments at 350ppm, 400ppm and 700ppm. Results are provided as climatological mean values over the forcing period (1974 – 2004). Atmospheric forcing, as per Qian et al. (2006), is identical between each of the 350ppm, 400ppm, and 700ppm runs.

RESULTS

Free Air Carbon Dioxide Enrichment Studies (FACE)

To investigate the range of responses of \( g_s \) across global sites (Fig. 1) we performed a synthesis of data from 51 FACE studies. Of the 1313 independent measurements across 52 species, 88.2% of the measurements showed a decrease in \( g_s \) in response to elevated CO\(_2\) (Fig. 2). However, 11.8% of the measurements showed an increase in \( g_s \) (Fig. 2). Such increases have gone largely unreported in the past, with most meta-analyses focusing on the overall mean negative response (decrease) of \( g_s \) to increasing CO\(_2\) concentration e.g. Ainsworth and Rogers (2007). Overall, \( g_s \) decreased by \(~\)19% on average across all FACE studies (Fig. 2).

Field survey of \( g_s \) responses to a 50ppm CO\(_2\) rise

A total of 51 C3 tree and shrub species (n = 209) were sampled during the in situ CO\(_2\) gas exchange measurements across four biomes (Fig. 3). Measurements reveal significant variation in the dynamic \( g_s \) responses to a \(~\)50ppm CO\(_2\) increase, which was selected to
represent anthropogenic climate change over the past 25 years (from 354 to 400 ppm) across
the different biomes (Fig. 3). The species of the boreal, temperate deciduous forest and
tropical seasonal forest (moist) biomes displayed an overall negligible response to increasing
CO₂ (Fig. 3). In contrast, the species of the tropical seasonal forest (dry) and, to an even
greater extent, the species of the tropical seasonal forest (drought), which had been subjected
to a one month long drought period prior to the measurements, displayed statistically
significant mean increases in gs in response to a 50 ppm rise in CO₂ (6.8% and 11.1%
respectively) (Fig 3). The grouping of stomatal responses between wet (i.e. boreal forest,
temperate deciduous forest, and tropical seasonal forest [moist]), and dry regions (i.e. tropical
seasonal forest [dry] and tropical moist seasonal forest [drought]) is also clearly reflected in
the corresponding changes in plant transpiration; decreasing and increasing mean
transpiration are observed respectively (Fig. 3).

*Field gs data – model comparison*

Our finding that gs can respond positively to increasing CO₂ is supported by the theoretical
predictions of the combined Farquhar-Ball-Berry (FBB) photosynthesis and gs model. The
model simulations, under a ~50ppm CO₂ rise scenario, demonstrate that increases in
atmospheric CO₂ drive increases in gs (Fig. 4) under conditions of high vapour pressure
deficit [VPD] (expressed as ea/ei in the model) and medium-high leaf temperature (Tv). The
dependence of gs responses to increasing CO₂ on air moisture and leaf temperature is also
observed in the field gas analysis data by positive correlations between gs responses and VPD
and leaf temperature (Fig. 5). This was also confirmed using mixed effects models, which
showed that the measured relative changes in gs are best explained when the relative changes
in A and ea/ei are used as fixed factors (AIC= 1633.8 Chisq= 4.0348, p= 0.044). The FBB
simulations provide a theoretical underpinning for the field observations by demonstrating
that plants can increase \( g_s \) as a response to increasing CO\(_2\), while simultaneously optimising WUE (Fig 4). In the model, increases in WUE are observed across all values of \( T_v \) and humidity. However, increases in WUE are highest in the parameter space where leaf humidity is low (dry regions) and \( T_v \) is high (warm-hot regions). A second simulation shows that the model produces an even higher \( g_s \) increase in response to a doubling of CO\(_2\) (to 700ppm) in dry and warm-hot regions of the parameter space (not shown).

To test how well the field Infrared-Gas-Analyser (IRGA) measured \( g_s \) is described by the FBB model, as well as the optimal \( g_s \) model of Medlyn et al. (2011), we used the recorded values of photosynthesis (A), \( T_v \) and water vapour concentration to calculate the model-implemented \( g_s \) of all 51 taxa analyzed. For the Medlyn et al. (2011) model we used published \( g_l \) values by Lin et al. (2015) for evergreen and deciduous trees and shrubs. Here \( g_0 \) values of 20 mmol m\(^{-2}\) s\(^{-1}\) are used. The comparison of modelled and recorded data revealed that the FBB model can accurately predict the observed \( g_s \), with the regression between estimated and observed \( g_s \) falling very close to the 1:1 line (Fig. 6). Furthermore, the model-implemented \( g_s \) responses are strikingly similar to those observed in the field (Fig. 3). A similar good fit was found when observed \( g_s \) values were plotted against the optimal \( g_s \) model of Medlyn et al. (2011) (Fig. S1).

The Community Land Model – a spatial investigation of global \( g_s \)

To gain a deeper understanding of the land-vegetation-system response to increases in CO\(_2\) at a spatial global scale, we performed simulations using the CLM4 land-vegetation model. The FBB model is also used for the parameterisation of CLM4. Simulations of the same CO\(_2\) increases in CLM4 resulted in a similar pattern of \( g_s \) responses (Fig. 7). In response to a 50ppm CO\(_2\) increase the CLM4 simulation produces predominantly negative changes
(decreases) in $g_s$ (Fig. 7). A ~3.2% annual global climatological maximum decrease in $g_s$ is simulated (Table 1). However, positive $g_s$ responses are also simulated, with a maximum increase of ~4.9% (Fig. 7, Table 1). A second annual global simulation, forcing the system with a doubling of CO$_2$ (to 700ppm), resulted in a larger ~16.8% global climatological maximum decrease in $g_s$ (Fig. 7). As in the 50ppm scenario, positive $g_s$ responses were also simulated across the low latitudes, this time with higher maximum positive changes of ~18.9% (Fig. 7, Table 1). There was a clear seasonal latitudinal and regional trend in the magnitude of $g_s$ change between months in the simulation (Fig. S2). For example, positive $g_s$ increases (to 50ppm) were mostly observed in the months between December to May in Central Africa and June to October in South Africa. In contrast, positive $g_s$ increases in Central America were observed in the months between January to June and in South America between June to November. Interestingly, the $g_s$ increases were accompanied by increases in soil moisture (Fig. 8, Table 1). Annual modelled regions experiencing the increasing $g_s$ response to CO$_2$ include countries such as Mexico, the Galapagos Islands, Dominican Republic, Columbia, Venezuela, Brazil, Bolivia, Sudan, South Sudan, Somalia, Tanzania, Democratic Republic of Congo (D.R.C.), Angola, Namibia, Botswana and Indonesia (Fig. 7, Table 2). Similar to our field observations, areas that showed positive $g_s$ increases were situated in hot and dry biomes (Table 2).

**DISCUSSION**

Overall, our results clearly demonstrate that in dry, warm environments, or during drought periods, plants can respond to increases in CO$_2$ by increasing their $g_s$, while, crucially, maximising the increase in their WUE (Figs. 3, 4, 7) compared to plants growing in the cooler moist conditions of the temperate latitudes. Implementation of the FBB model clearly shows a region of parameter space where CO$_2$, $g_s$ and WUE increases can coincide (Fig. 4).
The FBB model, when supplied with independently-measured values of \( V_{cmax} \), was able to accurately predict field observations, including the unexpected increases in \( g_s \) at high \( T_V \) and high VPD (Figs. 3, 6), a region of parameter space not often explored in standard gas analysis protocols which typically run under standardized temperatures and VPD of 22°C and 1kPa respectively. Although the measured \( g_s \) responses are small and difficult to capture under field conditions, Figs. 3 and 6 show excellent agreement between modelled and observed values and strongly support our claims.

For a more mechanistic understanding of the \( g_s \) responses documented above, we turn to a more detailed analysis of the FBB model. Firstly, we note that in the light-saturated conditions we are exploring here, \( A \) is Rubisco-limited and is thus expected to increase with temperature. In the particular formulation used here (see Materials and Methods), \( V_{cmax} \) increases roughly exponentially with temperature at temperatures below ~35°C, leading to a strong steepening of the \( A-c_i \) response curve as temperature increases (Fig. 4). This steepening carries over to the \( A-c_a \) response, as shown in Fig. 4; this figure also shows that higher humidity yields greater \( A \) at a given temperature and \( c_a \), because greater humidity promotes stomatal opening (Fig. 4) and thus greater \( c_i \), enhancing photosynthesis. Furthermore, we note that Equation (1) in the model (see Materials and Methods) implies that the sensitivity of \( g_s \) to ambient CO2, \( dg_s/dc_a \), at fixed temperature and humidity is given by:

\[
\frac{c^2 e^2}{m^4 p^2 e_i} \frac{\partial g_s}{\partial c_a} = \frac{c^2 \frac{\partial A}{\partial c_a}}{A \frac{\partial c_a}{\partial c_a}} - 1
\]  

Thus, increasing \( g_s \) in response to increasing \( c_a \) is possible when the first term on the right-hand side is greater than one, i.e. when the relative change in \( A \) is greater than the relative
change in $c_a$. This condition can be met when temperature is high and humidity is low (as exemplified by the solid circles in Fig. 4): in that case, $dA/dc_a$ is high while $A$ is low, bringing $dg_s/dc_a$ above zero (Fig. 4). When both temperature and humidity are high (squares in Fig. 4), $A$ is large enough to make the first term on the right less than one; conversely, when both temperature and humidity are low (triangles in Fig. 4), $A$ is low but $dA/dc_a$ is also low, and the first term on the right is still less than one.

In summary, the FBB model predicts $dg_s/dc_a > 0$ at high temperature and low humidity under light-saturated conditions because high temperature promotes a strong gain in $A$ per unit increase in $c_i$ (or $c_a$), while low humidity keeps the base value of $A$ low. Naturally, different model formulations would give quantitatively different results; in particular, the threshold values of temperature and humidity required for $dg_s/dc_a > 0$ are likely to be strongly model-dependent. However, the qualitative nature of the result appears robust, since increasing $V_{cmax}$ with increasing temperatures and stomatal opening with increasing humidity are both well-known features of plant physiology. Note in particular that the optimization models of Medlyn et al. (2011) also predict increasing $g_s$ as humidity increases (or VPD decreases), and would thus give qualitatively similar behaviour to the empirical Ball-Berry closure reported here (Fig. S1).

It is surprising that the possibility of $g_s$ increases as a response to rising CO$_2$ under these particular climatic conditions has not been highlighted before. As implied above, optimization models also predict similar increases within the CO$_2$ envelope tested in the present study (i.e. 354-400ppm CO$_2$) (Arneth et al. 2002; Konrad et al. 2008; Medlyn et al. 2013; Medlyn et al. 2011). For example, the optimization model of Konrad et al. (2008) demonstrates that the inflection point between rising and falling $g_s$ response to CO$_2$ is
dependent on the ‘cost of water’ (Fig. 4 in their article). In particular, high cost of water shifts
the inflection point to higher values, which are similar to those used in the present study.
These predictions fit well with both our measured and modelled gs responses.

It is intriguing that a substantial number of the FACE studies (see Materials and Methods)
also report increases in gs under super-ambient CO2. These increases in gs are mostly not
discussed, or are disregarded as methodological artefacts (Gunderson et al. 2002). Due to a
lack of standardised FACE protocols, the exact reasons why positive gs responses are
observed across these studies remain largely unclear. Possible reasons for the observed
increases might include; a) differences in the climatic and/or cuvette measurement conditions;
b) differences in soil nutrient and water status; c) differences in the signal to noise ratio with
regard to gs (i.e. species with low gs show a greater propensity for erroneous measurements);
d) studies do not consistently record the time when measurements are taken, despite literature
which shows that gs responses to CO2 are highly dependent on the time of day (Konrad et al.
2008). Unfortunately, FACE studies inherently include a range of weather regimes/cuvette
conditions and measurement times, which are inconsistent amongst studies and typically
unreported. It is therefore not possible to assess the role of these conditions with regard to the
reported gs increases. Secondly, nutrient concentrations and soil water content naturally vary
between sites, but are inconsistently documented across studies [e.g. Naumburg et al. (2003)]
making direct comparison unfeasible at this time. Regarding the potential low signal to noise
ratio of the species that display increases in gs as a response to increased CO2, our meta-
analysis of FACE studies showed that there is no significant difference in the gs values
between species that show either positive or negative responses to CO2 (F=1.663, p=0.198).
The same was found to be the case for the gs responses of different PFTs, with the exception
of shrubs (F=4.122, p<0.001). Thus, the observed positive gs responses in FACE studies may
arise for a number of different reasons. If we were to speculate, it is likely that at least part of
them are due to warm, dry conditions, as demonstrated by our field data (Fig. 3, 5) and model
comparisons (Fig. 6 and Fig. S1).

Positive $g_s$ responses have the potential to alter regional or even global hydrological and
carbon cycles, and other ecological processes. We acknowledge that there are limitations in
assessing long term $g_s$ trends through field measurements, as they cannot account for long
term water availability changes resulting from the CO$_2$ effects on $g_s$. Several studies have
shown that decreasing soil moisture can elicit greater stomatal closure under elevated CO$_2$
than ambient CO$_2$ (Leakey et al., 2006; Piao et al., 2007; Gray at al., 2016). Similarly,
increases in LAI has been shown to reduce soil moisture, thus indirectly affecting $g_s$ (Field et
al. 1995; Wenfang et al. 2013). Our global simulations using CLM can only partially test for
this, as LAI was not simulated here. It also needs to be noted that current CLM
parameterizations do not account for many morphological plant responses to elevated CO$_2$
(e.g. changes in stomatal density). Keeping these reservations in mind and although
predictions of future $g_s$ are somewhat beyond the scope of the present study, Fig. 8 shows that
in regions where $g_s$ is predicted to increase in response to a 50 and 350ppm CO$_2$ rise, soil
moisture also increases (in this instance the increased soil moisture may be caused by water
savings due to suppressed $g_s$ in prior months, and may in fact cause the annual mean increase
of $g_s$ at these locations). Coupled with potential increases in LAI in response to elevated CO$_2$
(Frank et al. 2015; Niu et al. 2013; Piao et al. 2007; Schymanski et al. 2015; Wu et al. 2012),
regionally increasing $g_s$ may act to offset the much studied effects of decreasing $g_s$ e.g.
increasing river runoff (Betts et al. 2007; de Boer et al. 2011; Gedney et al. 2006;
Gopalakrishnan et al. 2011; Lammertsma et al. 2011), or even drive enhanced drought and
desertification in certain regions (Dai 2013). Areas that were predicted by CLM to show
increases in $g_s$ with elevated CO$_2$ (~50 and 350ppm) are located in hot and dry biomes (Fig. 7 and Table 2). A monthly analysis of $g_s$ for CLM also suggests that the relative timing of temperature and relative humidity is important in driving the $g_s$ increases; which leads us to expect increases in $g_s$ in monsoonal regions (Fig. S2). However, due to other confounding factors (e.g. vegetation types and/or soil moisture) this expectation is not always met (e.g. India) and requires further investigation which is beyond the scope of the current study.

Continued land-vegetation model development based on field data at the biome (and community-species) level, as well as further Earth System Model inter-comparison studies, will be required to assess the implications of this shift in our understanding of vegetation responses to elevated CO$_2$, and for improved prediction of the global hydrological cycle, particularly in dry and warm-hot regions.

We demonstrated that increases in $g_s$ can occur under elevated CO$_2$ in environments that are hot and dry (high VPD). Our field observations across several global biomes are in excellent agreement with predictions from optimization models and fall within a previously unrecognised parameter space within the FBB model. The implications of our findings are of global significance for future modelling of soil-vegetation-climate feedbacks, as the FBB model is also implemented in CLM. Although the majority of the global vegetation respond by decreasing $g_s$ under elevated CO$_2$, biomes that already experience drought conditions are likely to show increases in $g_s$. It remains to be seen how these increases will affect soil-canopy-atmosphere climate feedbacks in the future, particularly in areas that are already expected to be more threatened as a result of predicted changes in climate.

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**SUPPLEMENTAL MATERIAL** (see separate files)

**Table S1.** Species list and site description

**Figure S1.** Comparison of measured and modelled gs values under 354 and 400ppm of atmospheric CO₂ using the optimal gs model by Medlyn *et al.* (2011).
**Figure S2.** Stomatal conductance response to increasing CO$_2$ in the CLM4 land-vegetation model for each month of the year. Negative and positive $g_s$ responses to increasing CO$_2$ in CLM4 (400ppm relative to 350ppm).

**FIGURE LEGENDS**

**Figure 1.** The location of FACE studies included in our assessment. 51 FACE studies are shown (most overlap on this scale). Most FACE studies are located in Northern hemisphere locations between 30-60° North. FACE studies which did not, to our knowledge, document $g_s$ changes were not included. See Materials and Methods for all cited studies used.

**Figure 2.** FACE synthesis of $g_s$ responses to increasing CO$_2$ concentration. Kernel density probability distribution of the percentage change of $g_s$ to increasing CO$_2$ concentration. Each colour represents a different Plant Functional Type (PFT). The percentage $g_s$ change is expressed as the delta change of $g_s$ between ambient and high CO$_2$ treatments. Solid lines are median (blue) and mean (red) change in $g_s$ across all PFTs. The dashed line is the zero percentage change mark. See Materials and Methods for details of the synthesis and cited FACE studies used.

**Figure 3.** Dynamic $g_s$ responses to a subtle CO$_2$ increase across four biomes observed in field conditions compared with modelled responses. A, Percentage change in $g_s$ during the transition from 354 (sub-ambient) to 400ppm (modern ambient) atmospheric CO$_2$, which is representative of atmospheric changes that have occurred over the past ~25 years. The boxes signify the distribution of the 25%–75% quartiles, with median and average values represented by a vertical line and an open square within the box, respectively. The whiskers indicate the distribution of the 5–95% quartiles. Solid boxes represent the field...
measurements. Stripped boxes represent the modelled percentage responses of \( g_s \) using the Farquhar-Ball-Berry model and the \( A, T_V \) and \( e_o/e_i \) values measured in the field. Different letters denote statistically significant differences between biomes (\( p \leq 0.05 \)). Asterisks indicate within-biome statistically significant differences between the conductance values at 354 and 400 ppm of CO\(_2\). N= 24-66 independent measurements depending on biome (see Table S1 for species list). B, Percentage change in transpiration between 354 and 400 ppm atmospheric CO\(_2\). C, Locations of expedition sites visited during this study. See Table S1 for geographical coordinates and site information.

Figure 4. Results from the Farquhar-Ball-Berry combined photosynthesis and \( g_s \) model.

A, \( A-c_i \) response curves at two different leaf temperatures, as indicated in the legend. B, \( A-c_a \) response curves at two different temperatures and humidities (see legend in panel C). C, Sensitivity of \( A \) to \( c_a \), normalized by \( A/c_a \), as a function of \( c_a \) at two different temperatures and humidities, as indicated by the legend. D, Predicted \( g_s \) at \( c_a = 350 \) ppm as a function of leaf temperature and humidity. E, Predicted percentage change in \( g_s \) when \( c_a \) changes from 350 to 400 ppm, with zero contour highlighted by solid black line. F, Predicted percentage change in water use efficiency WUE when \( c_a \) changes from 350 to 400 ppm. Symbols in all panels indicate three selected cases: high temperature, low humidity (circles); high temperature, high humidity (squares), and low temperature, low humidity (triangles).

Figure 5. Gas analysis relationship between \( g_s \) and vapour pressure deficit and leaf temperature. Linear relationship and 95% confidence bands (dotted lines) between the percentage change in \( g_s \) during the transition from 354 (sub-ambient) to 400 ppm (modern ambient) atmospheric CO\(_2\) and A, VPD (kPa) (\( y=5.94x-5.24, r^2=0.21, p<0.01 \)) and B, leaf temperature (\( ^\circ C \)) (\( y=0.63x-12.82, r^2=0.14, p<0.01 \)). Data represent species averages with an
average number of four individuals measured per species.

Figure 6. Comparison of measured and modelled $g_s$ values under 354 and 400ppm of atmospheric CO$_2$. Relationship (0.95x+6.8, $r^2=0.431$, solid line) between measured and modelled $g_s$ values. Stomatal conductance was modelled using the Farquhar-Ball-Berry model and the $A, T_v$ and $e_a/e_i$ values measured in the field. The dashed line represents the 1:1 relationship. Mixed effects model results showed that the relative changes in $g_s$ are best explained when the relative changes in $A$ and $e_a/e_i$ are used as fixed factors (AIC= 1633.8 Chisq= 4.0348, p= 0.044).

Figure 7. Annual $g_s$ response to increasing CO$_2$ in the CLM4 land-vegetation model. Negative and positive $g_s$ responses to increasing CO$_2$ in CLM4, for A, a 400ppm and B, a 700ppm scenario, relative to 350ppm. Modelled regions experiencing positive $g_s$ responses for both A, and B, include parts of Central America, South America, Africa and Asia (see Table 2 for more detail). It should be noted that the majority of the land surface experiences decreases in $g_s$ in response to increasing CO$_2$.

Figure 8. Detailed analysis of CLM grid cells showing positive $g_s$ responses under a 400 and 700ppm CO$_2$ scenario. Percentage change of soil moisture and $g_s$ for a 400ppm (solid lines) and a 700ppm (dashed lines) scenario, relative to 350ppm. Only grid cells that showed positive increases in $g_s$ are used for this analysis (geographical areas coloured in reds and oranges in Fig. 7).
Table 1. CLM maximum annual increases/decreases and percentage of grid cells showing increases/decreases or no change in $g_s$ and soil moisture across the globe.

<table>
<thead>
<tr>
<th>$\text{CO}_2$ (ppm)</th>
<th>Variable</th>
<th>Max. decreases</th>
<th>Max. increases</th>
<th>Percentage no. of grid cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
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<tr>
<td>400-350</td>
<td>Stomatal conductance (s/m)</td>
<td>0.00075</td>
<td>0.00004</td>
<td>1.94</td>
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<td></td>
<td>Soil moisture (kg/m$^2$)</td>
<td>0.1</td>
<td>1.1</td>
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<tr>
<td>700-350</td>
<td>Stomatal conductance (s/m)</td>
<td>0.00004</td>
<td>0.00001</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>Soil moisture (kg/m$^2$)</td>
<td>2.6</td>
<td>0.01</td>
<td>80.87</td>
</tr>
</tbody>
</table>

Table 2. Countries and associated biomes that showed an annual positive increases in $g_s$ under a 50ppm increase in $\text{CO}_2$.

<table>
<thead>
<tr>
<th>Continent</th>
<th>Country</th>
<th>Biome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central America</td>
<td>Mexico</td>
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<tr>
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<td>Galapagos</td>
<td>Mediterranean Forests, Woodland &amp; Shrub</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Dominican</td>
<td>Tropical &amp; Subtropical Dry Broadleaved Forest &amp;</td>
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<tr>
<td>South America</td>
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<td>Deserts &amp; Xeric Shrublands</td>
</tr>
<tr>
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<td>Venezuela</td>
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<td>Brazil</td>
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<tr>
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<td>Sudan</td>
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<tr>
<td>Region</td>
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<td>--------</td>
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<td>Asia</td>
<td>Indonesia</td>
<td>Tropical &amp; Subtropical Dry Broadleaved Forest</td>
</tr>
</tbody>
</table>

1
Observed $g_s$ (mmoles m$^{-2}$ s$^{-1}$) vs. Modeled $g_s$ (mmoles m$^{-2}$ s$^{-1}$). The plot shows different biomes: Boreal forest, Temperate deciduous forest, Tropical seasonal forest (moist), Tropical seasonal forest (drought), and Tropical seasonal forest (dry). A linear regression line is drawn with the equation $y = 6.8 + 0.95x$, and the coefficient of determination $r^2 = 0.431$. The data points are color-coded according to the biomes.